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(54) Title: METHOD FOR PRODUCING CIRCULAR OR MULTIMERIC PROTEIN SPECIES *IN VIVO* OR *IN VITRO* AND RELATED METHODS

(57) Abstract: A method is disclosed for the *in vitro* or *in vivo* cyclization of protein or peptide sequences. Also disclosed is a method of fusing polypeptide sequences while bound to a solid support. These protein manipulation techniques relied on the *trans*-splicing activity of a split intein, such as the naturally occurring split intein from the *dnaE* gene of *Synechocystis* sp. PCC6803 (*Ssp DnaE* intein). The cyclization procedures required the fusion of C- and N-terminal intein splicing domains to the N- and C-termini, respectively, of a target protein (Intein_C-target protein-Intein_N). Cyclization *in vivo* occurred post-translationally when the two complementary intein splicing domains ligated the N- and C-terminus of the target protein. *In vitro* cyclization also utilized and Intein_C-target protein-Intein_N precursor protein, in which the intein domains were fused to a chitin binding domain (CBD). Protein expression was conducted under conditions that favored the accumulation of precursor protein, which was immobilized on a chitin resin. The circular protein species were eluted from the chitin resin following incubation under conditions that favored protein splicing. *Trans*-splicing was used to ligate polypeptides on a solid support by generating a protein composed of a CBD fused to a C-terminal intein splicing domain and target protein (1). This was incubated with a protein composed of target protein (2) fused to an N-terminal intein splicing domain and a CBD. The precursor proteins were immobilized on a chitin resin where *trans*-splicing resulted in the ligation of target protein (1) to target protein (2). These techniques greatly expand the procedures available for protein engineering and modification.

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